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〔Article〕

Action Mechanism of Iridoid Compounds on Guinea-pig Right Atrium Specimens

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Abstract

We examined the actions of iridoid compounds (aucubin (Auc), geniposidic acid (GA)) and a non-iridoid compound (chlorogenic acid (CA)) contained in Eucommia leaves [1] [2], which show blood pressure-lowering effects, on the heart using right atrial specimens isolated from guinea pigs. These 3 compounds showed negative inotropic effects (NIE) and negative chronotropic effects (NCE) at a final concentration of 10^{-5} or 10^{-4} M in an experiment using right atrial specimens. Furthermore, pretreatment with 10^{-5} M atropine (Atr) led to the disappearance of the NIE of Auc and GA. This suggests that the enhancement of the parasympathetic nerves is involved in the action mechanism of iridoid compounds, as indicated for acetylcholine (ACh). An experiment regarding the dose-response curve (DRC) of ACh using guinea-pig intestines also demonstrated that this substance acted on the same receptors. On the other hand, the NIE of CA, as a non-iridoid compound, did not disappear despite pretreatment with 10^{-5} M Atr, resembling the actions of Ca antagonists. Concerning rabbit blood pressure responses, the administration of CA at 1 mg/kg decreased the blood pressure, but GA did not reduce it, suggesting that the blood pressure-lowering effects of Eucommia leaves are associated with the Ca antagonism of CA.

Keywords: Iridoid, aucubin, geniposidic acid, chlorogenic acid, negative inotropic effect (NIE), negative chronotropic effect (NCE), heart rate (HR)

植物成分イリドイド化合物のモルモット右心房標本に対する作用機序の解明

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1）名古屋学院大学 / 2）金城学院大学

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1. Introduction

Eucommia is the bark of a dioecious, deciduous tree, *Eucommia ulmoides* Oliver (place of origin: Sichuan, China). It has been used to prepare tonics, analgesic drugs, sedative drugs, hypotensive drugs, and diuretics over many years. In the oldest Chinese herbal book, "Shennong Ben Cao Jing" (1600 years before), it was classified as good quality [1]. Tagawa et al. reported that the methanol fraction of a Eucommia leaf extract exhibited hypotensive actions, acting on muscarinic acetylcholine receptors [2]. The principal components of Eucommia leaves are geniposidic acid (GA) and chlorogenic acid (CA). However, no study has reported the actions of these compounds on muscarinic acetylcholine receptors.

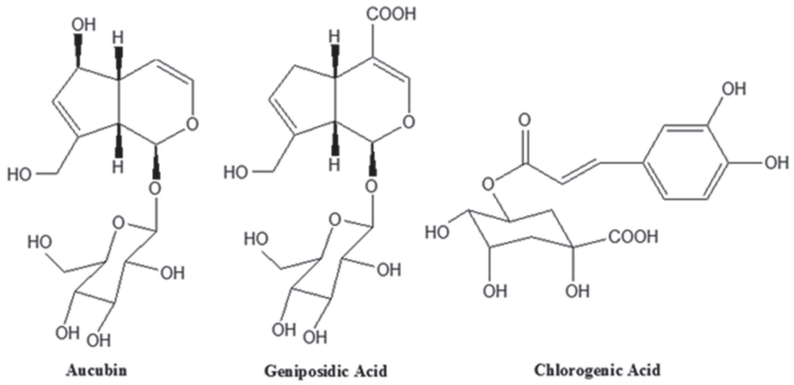


Fig. 1. Structural formulae of iridoid compounds, aucubin (Auc) and geniposidic acid (GA), and a non-iridoid compound, chlorogenic acid (CA)

In this study, we examined the actions of iridoid compounds (aucubin (Auc), GA) and a non-iridoid compound (CA) contained in Eucommia leaves on the heart using right atrial specimens isolated from guinea pigs. These compounds showed negative inotropic effects (NIE) and negative chronotropic effects (NCE) at 10^{-4} M. In addition, these compounds were intravenously administered to rabbits to investigate the blood pressure-lowering action mechanism.

2. Materials and Methods

2.1 Experiment of the heart

Guinea pigs (male, body weight: 320 to 350 g) were sacrificed under ether anesthesia. Immediately, thoracotomy was performed, and the pulsating heart was extirpated and placed in Krebs-Henseleit (K-H) solution (pH: 7.4), which was saturated with mixed gas (95%O_2, 5%CO_2), to prepare a specimen of the right atrium. Its end was fixed to a portion of the mixed gas insertion tube

of a Magnus device with thread, and the other end was connected to the end of an FD pickup system (Nihon Kohden Corp., TB-612T) with thread. The volume of K-H solution in the Magnus tank was established as 20 mL, and 0.5 g resting tension was added to the atrium. Autonomic pulsation-related tension was converted to voltage through a strain-gauge-type tensiometer, and recorded through an amplifier (Nihon Kohden Corp., RP-5).

2.2 Experiment using ileum specimens

After removing the guinea-pig heart, the small intestine was extirpated, and placed in Tyrode solution (30°C), which was saturated with oxygen, to prepare an intestinal specimen. Its end was fixed to a portion of the air insertion tube of a Magnus device with thread, and the other end was connected to the end of a displacement transducer (Nihon Kohden Corp., TD-111T) with thread. The volume of Tyrode solution in the Magnus tank was established as 20 mL, and 1.0 g resting tension was added to the intestinal tract. Nutrient solution was agitated with air.

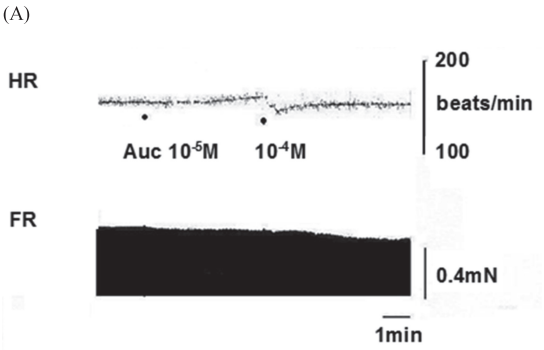
2.3 Blood pressure reactions of rabbits

Arterial cannulae were inserted into the cervical arteries of rabbits anesthetized with urethane (male, body weight: 2.5 kg) to measure the blood pressure through an amplifier (Nihon Kohden Corp., AP-601G). Simultaneously, the heart rate was also measured through an amplifier (Nihon Kohden Corp., AT-601G). Each drug was infused through the ear vein.

3. Results

3.1 Effects of Auc on right atrial specimens isolated from guinea pigs

The administration of an iridoid compound, Auc (10^{-5} M), decreased the heart rate (HR) and contractility (Fc). When administering 10^{-5} or 10^{-4} M Auc after pretreatment with 10^{-5} M atropine (Atr), there were no such actions (Fig. 2).



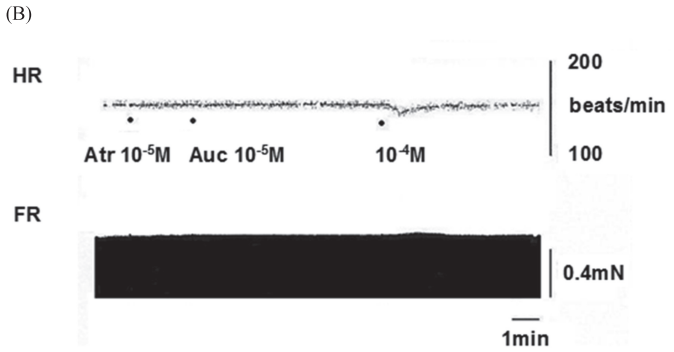


Fig. 2. Effects of Auc on right atrial specimens isolated from guinea pigs

3.2 Effects of GA on right atrial specimens isolated from guinea pigs

The administration of an iridoid compound, GA (10^{-5} M), decreased the HR and Fc. When administering 10^{-5} or 10^{-4} M GA after pretreatment with 10^{-5} M Atr, there were no such actions (Fig. 3).

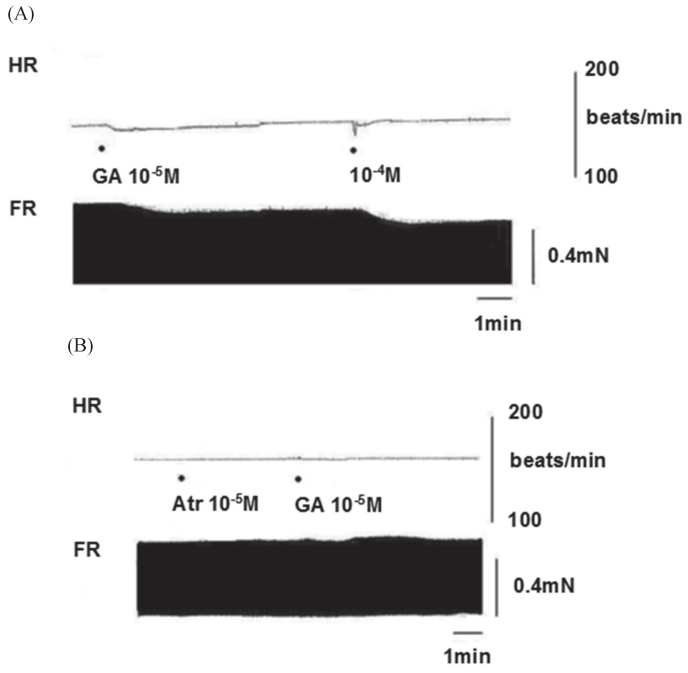


Fig. 3. Effects of GA on right atrial specimens isolated from guinea pigs

3.3 Effects of CA on right atrial specimens isolated from guinea pigs

The administration of a non-iridoid compound, CA (10^{-5} M), decreased the HR and Fc. Even when administering 10^{-5} or 10^{-4} M CA after pretreatment with 10^{-5} M Atr, there were decreases in the HR and Fc (Fig. 4).

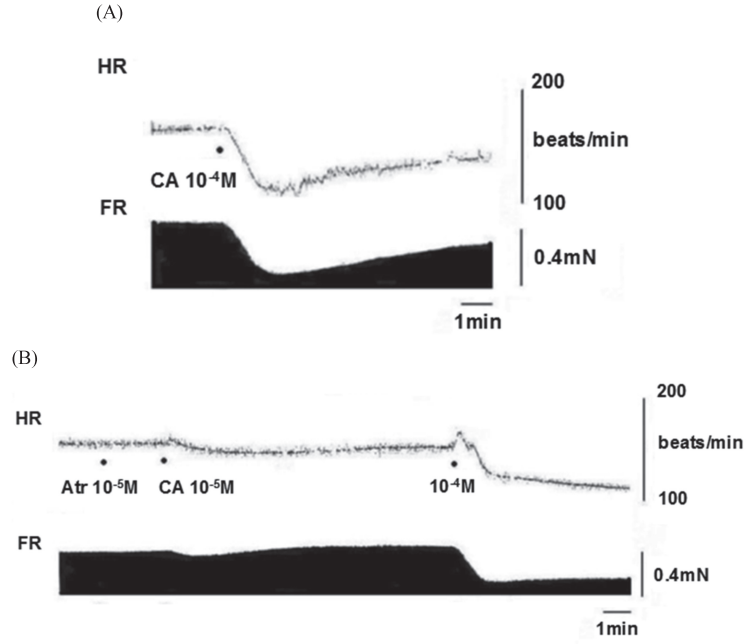


Fig. 4. Effects of CA on right atrial specimens isolated from guinea pigs

3.4 Effects of GA and CA on the intestinal tracts extirpated from guinea pigs

The effects of acetylcholine (ACh), 10^{-4} M GA + ACh, and 10^{-4} M CA + ACh on the intestinal tracts extirpated from guinea pigs are shown in Fig. 5. Pretreatment with an iridoid compound, 10^{-4} M GA, led to the parallel shift of the dose-response curve (DRC) of ACh to the right, showing competitive antagonism; the two agents may act on the same receptors. The pA_2 value was 7.52 ± 0.08 ($n = 5$). On the other hand, pretreatment with a non-iridoid compound, 10^{-4} M CA, decreased a maximum concentration to $87.4 \pm 3.0\%$, showing non-competitive antagonism; another receptor may be involved. The pD'_2 value was 3.17 ± 0.08 ($n = 5$) (Fig. 5).

3.5 Effects of GA and CA on the blood pressure reactions of rabbits

The results of the experiment regarding rabbit blood pressure reactions to an iridoid compound, GA, and a non-iridoid compound, CA, are presented in Fig. 6.

After the administration of CA at 1 mg/kg, the rabbit blood pressure decreased, as indicated for a Ca antagonist, verapamil. On the other hand, there was no decrease in the blood pressure after the

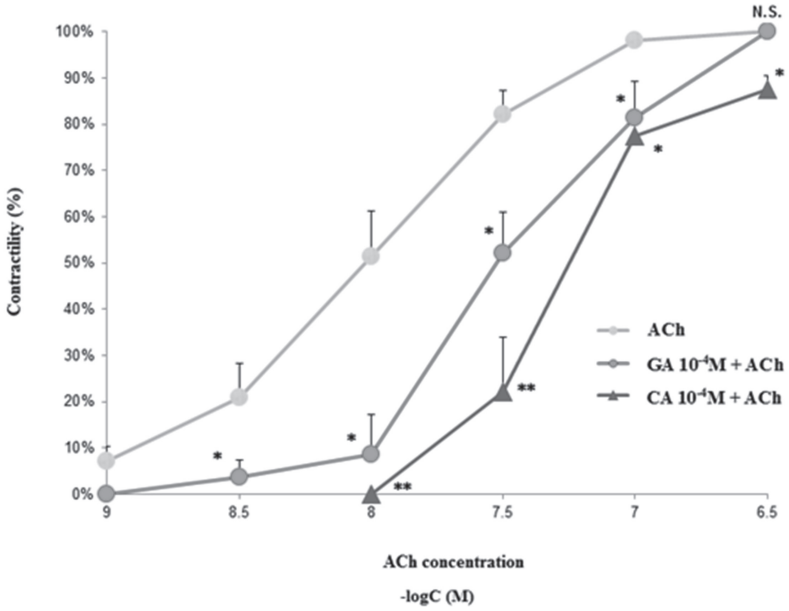


Fig. 5. Effects of GA and CA on the intestinal tracts extirpated from guinea pigs
Contractility (%), ACh concentration
Data are expressed as the mean values of 5 preparations ± S. E. M; N. S.; *p<0.05; **p<0.01.
(ACh *VS* GA 10^{-4}M+ACh, CA 10^{-4}M+ACh).

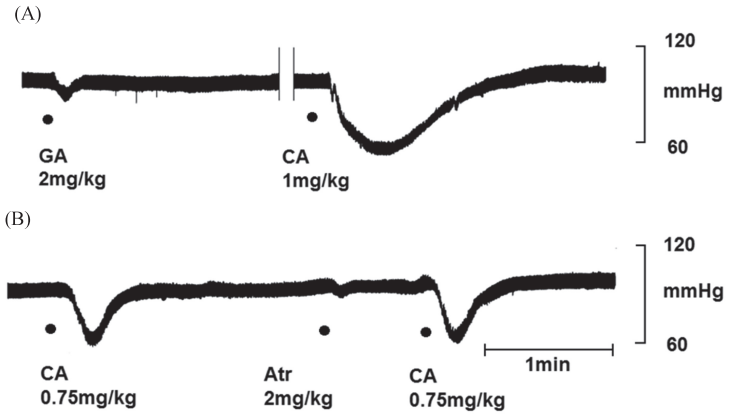


Fig. 6. Effects of GA and CA on rabbit blood pressure reactions

administration of GA at 1 mg/kg. When increasing the dose to 2 mg/kg, GA slightly decreased the blood pressure, but the rate of decrease corresponded to approximately 1/30 of the area under the curve for blood-pressure fall related to CA at 1 mg/kg.

4. Discussion

Iridoid compounds, Auc and GA, and a non-iridoid compound, CA, showed negative inotropic and chronotropic effects on guinea-pig right atrial specimens at final concentrations of 10^{-5} and 10^{-4} M. Pretreatment with a parasympathetic nerve blocker, Atr (10^{-5} M), led to the disappearance of the actions of these iridoid compounds. This suggests that the iridoid compounds exhibited NIE through the enhancement of the parasympathetic nerves, as reported for ACh. Furthermore, the experiment regarding the DRC of ACh calculated for the guinea-pig intestinal tracts showed that these substances acted on the same receptors.

On the other hand, the NIE of a non-iridoid compound, CA, were not inhibited by pretreatment with 10^{-5} M Atr, and the DRC experiment showed non-competitive antagonism, suggesting that the action mechanism differs. CA exhibited actions similar to those of Ca antagonists. Although the NCE of these compounds may be involved in the blood pressure-lowering effects of Eucommia leaves, blood pressure actions are expressed as V = R • I (blood pressure = vascular resistance x cardiac output), and the vascular resistance, rather than the cardiac output, influences the value; therefore, concerning the action mechanism of CA, which shows Ca antagonistic actions, its smooth muscle-dilating actions may be closely involved in the blood pressure-lowering effects of Eucommia leaves. In the experiment regarding rabbit blood pressure reactions, CA at 1 mg/kg decreased the blood pressure, but GA did not reduce it. According to a study, Eucommia leaf glycosides, as inherent components, such as GA, contained in the leaves, activated vascular endothelium-derived relaxing factor (NO), and acted on the parasympathetic nerves (muscarinic receptors), relaxing the smooth muscle, reducing the blood flow resistance, and decreasing the blood pressure [2]. However, the above finding suggests that the Ca antagonistic actions of CA are involved in the blood pressure-lowering action mechanism. Furthermore, diarrhea, as an adverse reaction, may be associated with ACh-like actions on the smooth muscle of the digestive tract.

5. Conclusion

Pretreatment with 10^{-5} M Atr led to the disappearance of the NIE of Auc and GA on guinea-pig right atrial specimens, suggesting that the enhancement of the parasympathetic nerves is involved in the action mechanism of iridoid compounds, as indicated for ACh. The experiment regarding the DRC of ACh calculated for the guinea-pig intestinal tracts also showed that these substances acted on the same receptors. On the other hand, the NIE of CA, as a non-iridoid compound, did not disappear despite pretreatment with Atr, resembling the actions of Ca antagonists. Concerning rabbit blood pressure responses, the intravenous administration of CA at 1 mg/kg decreased the blood pressure, as reported by Cheng et al. [3], but GA did not reduce it even at 2 mg/kg, suggesting that the blood

pressure-lowering effects of intravenously administered CA are associated with the Ca antagonism.

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References

[1] Japanese Eucommia Research Group http://www.eucommia.gr.jp/index.html

[2] Tagawa C, Nakazawa Y, Tagashira E, Ueda T, Yamaguchi Y, Ohara T, Onizuka S, Nishibe S. Effect of Eucommia Leaf (*Eucommia ulmoides* Oliver; Du-Zhong yge) Extract on Blood Pressure (2). Nature Medicines 2005; 59(3): 117–120.

[3] Cheng C. J. T, Lee Y. Y, Hsu F. L, Chang W, Niu C. S. Chinese Pharmaceutical Journal 1994; 46: 575–582.